The Plant Cell, Vol. 4, 617, June 1992

IN THIS ISSUE

# Cutinase—Not a Weapon in Fungal Combat?

Some of the first lines of defense a plant has against potential pathogen invaders are its external cuticle layer and the walls of its cells. To invade a plant with any success, a pathogen must first pass through these barriers. Some pathogens, including fungi, use mechanical means to muscle their way through the protective layers, whereas other pathogens are able to enter plants only at sites, such as wounds, where the barriers have already been breached. Still other pathogens have been proposed to gain entry to plant cells by enzymatically degrading cuticle or cell walls. Recent gene knockout studies have, however, begun to call into question the importance of these enzymes for fungal pathogenicity. In this issue, Stahl and Schäfer (pages 621–629) show that *Nectria haematococca*, which causes foot rot of pea, is just as pathogenic when it lacks a functional cutinase gene as when it has one.

Until recently, cutinase seemed certain to be essential for the pathogenicity of this fungus. *Fusarium solani* f sp *pisi*, the asexual form of *N. haematococca*, secretes an extracellular cutinase and can grow on cutin as a sole carbon source (for review, see Kolattukudy, 1985). The purified cutinase is a serine hydrolase that is specific for primary alcohol esters, the dominant ester linkage in cutin. The presence of cutin induces the accumulation of the cutinase mRNA (Woloshuk and Kolattukudy, 1986), possibly by direct activation of cutinase gene transcription by cutin monomers (Podila et al., 1988). By contrast, glucose represses cutinase mRNA accumulation. Both antibody and chemical inhibitor studies suggested that cutinase is essential for fungal pathogenicity: fungal spores placed on pea stems in the presence of an anti-cutinase antibody or serine hydrolase inhibitors such as diisopropyl fluoryl phosphate were no longer pathogenic.

The results of genetic studies appeared to confirm the conclusions from these functional studies. For instance, field isolates of *F. solani* f sp *pisi* with low cutinase activity also had reduced virulence. Moreover, in a screen for UV-induced cutinase-deficient mutants, Dantzig et al. (1986) isolated a mutant that produced only 10 to 20% of the normal cutinase specific activity and showed a concomitant decrease in pathogenicity in the pea stem bioassay. Not only that, but cutinase endows at least one fungus that can normally infect only wounded sites with the ability to penetrate intact cuticle: Dickman et al. (1989) transferred the *F. solani* cutinase gene into *Mycosphaerella*, a fungus that infects wounded papaya fruit, and found that cutinase-expressing transformants could form lesions on nonwounded papaya fruit.

Together, these results supported the following simple scenario (Kolattukudy, 1985). When *F. solani* spores contact cuticle, the basal cutinase activity catalyzes the formation of a small amount of cutin monomers. The germinating spores sense the presence of these monomers and, in response, activate cutinase gene transcription to high levels. The abundance of cutinase then digests away the cuticle in the vicinity of the fungus, allowing hyphae to invade the plant.

To test directly whether the correlation between cutinase activity and pathogenicity reflects an absolute requirement for cutinase in the infection process, Stahl and Schäfer used gene replacement techniques to disrupt the single cutinase gene of a highly virulent strain of *N. haematococca*. They constructed a plasmid that contains a selectable marker (the *hph* gene, which confers resistance to hygromycin B) in place of the internal region of the cutinase gene, transformed the fungus with this plasmid, and selected for hygromycin-resistant transformants. DNA gel blot analysis indicates that in some transformants the disrupted gene occupies the position formerly occupied by the wild-type gene. In other transformants, a single recombination event caused the plasmid to integrate next to the native gene. Still another class of transformants arose when the plasmid inserted at distant locations in the genome.

RNA and protein analyses confirm that the replacement of the wild-type gene with the disrupted gene generated a null allele: neither cutinase mRNA nor the protein itself nor any cutinolytic activity are detectable in the mutant. In addition, although null mutants grow normally on a rich medium, they grow poorly when cutin is the sole carbon source—further evidence that they lack cutinase.

Despite the evident loss of the cutinase gene product, null mutants are as pathogenic as the virulent strain from which they were derived, both in a pea stem bioassay and when inoculated as conidia into the soil surrounding pea plants. Stahl and Schäfer speculate that the previous experiments from which a requirement for cutinase was inferred may have been misleading. For example, the antibodies and chemical inhibitors may not have been as specific for cutinase as had been thought, and the low virulence of cutinase-deficient variants may have resulted from lesions in genes other than the cutinase gene. Stahl and Schäfer also point out that their results do not completely rule out a requirement for cutinase in infection: a cutinase of a different sequence would not have been knocked out, and, if such a cutinase is not cutin activated in vitro (Stahl and Schäfer's deletion mutant can barely grow on cutin), it would have escaped detection.

Stahl and Schäfer's results are particularly interesting in the light of the results of Scott-Craig et al. (1990), who investigated the role of the cell wall-degrading

enzyme endopolygalacturonase (endoPG) in pathogenicity of the maize pathogen *Cochliobolus carbonum.* PG is one of several enzymes that depolymerize pectin, and endoPG is thought to act early in the infection process. Mutagenesis studies had hinted that PGs might not be essential in fungal pathogenicity, but in no case had a specific genetic lesion been identified. By transforming *C. carbonum* with a plasmid containing a fragment of the endoPG gene and a selectable marker, Scott-Craig et al. selected transformants in which the entire plasmid had integrated at the endoPG locus, disrupting the native gene. Although such mutants can grow on pectin as a sole carbon source and, in fact, retain about a quarter of the normal level of PG activity, end group analysis indicated that the residual PG activity is due solely to exoPG. Mutants that lack endoPG nevertheless remain virulent on maize. Although this is strong evidence that *C. carbonum* does not require endoPG to attack maize and may not even have to degrade the cell wall, it is possible that an undetected endoPG of divergent sequence or the exoPG provides the necessary measure of enzymatic degradation.

If these enzymes are not necessary for pathogenicity, then what might their role be? In addition to being a pathogen, *N. haematococca* lives as a saprophyte, and, as Stahl and Schäfer point out, a cutin-inducible cutinase would allow this fungus to degrade cutin in plant debris. These fungi presumably do possess specific pathogenicity factors of some sort, and more genetic experiments will be required in these and other pathogenic fungi to pin down the actual requirements for pathogenicity. In the meantime, the results presented in this issue suggest that despite earlier indications to the contrary, enzymatic degradation of the cuticle and cell wall may not be an important means by which plant pathogenic fungi break through the plant's defenses.

**Rebecca Chasan**

## REFERENCES

**Dantzig, A.H., Zuckerman, S.H., and Andonov-Roland, M.M.** (1986). Isolation of a *Fusarium solani* mutant reduced in cutinase activity and virulence. J. Bacteriol. **168,** 911–916.

**Dickman, M.B., Podila, G.K., and Kolattukudy, P.E.** (1989). Insertion of cutinase gene into a wound pathogen enables it to infect intact host. Nature **342,** 446–448.

**Kolattukudy, P.E.** (1985). Enzymatic penetration of the plant cuticle by fungal pathogens. Annu. Rev. Phytopathol. **23,** 223–250.

**Podila, G.K., Dickman, M.B., and Kolattukudy, P.E.** (1988). Transcriptional activation of a cutinase gene in isolated fungal nuclei by plant cutin monomers. Science **242,** 922–925.

**Scott-Craig, J.S., Panaccione, D.G., Cervone, F., and Walton, J.D.** (1990). Endopolygalacturonase is not required for pathogenicity of *Cochliobolus carbonum* on maize. Plant Cell **2,** 1191–1200.

**Woloshuk, C.P., and Kolattukudy, P.E.** (1986). Mechanism by which contact with plant cuticle triggers cutinase gene expression in the spores of *Fusarium solani* f. sp. *pisi.* Proc. Natl. Acad. Sci. USA **83,** 1704–1708.